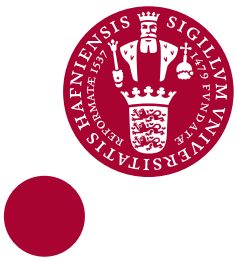

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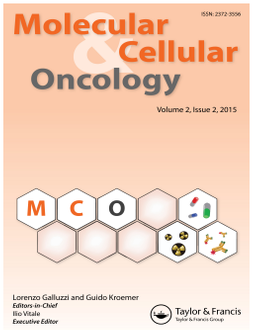

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The retinoblastoma protein: multitasking to suppress tumorigenesis

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Tumor suppressor activity of the retinoblastoma protein pRB is preserved despite loss of interaction with E2F transcription factors (E2F) or proteins harboring a leucine-x-cysteine-x-glutamic acid motif (LxCxE, where x is any amino acid). This indicates that pRB uses several parallel pathways to suppress tumorigenesis, which may also include E2F- and LxCxE-independent interactions.

A frequent event in tumor development is loss of the retinoblastoma tumor suppressor gene, *RB1*, or altered activity of upstream regulators that inactivate its product, the retinoblastoma protein (pRB). pRB and its 2 family members, p130 and p107, collectively known as pocket proteins, play key roles during the G_1 phase of the cell cycle, when they inhibit the E2F family of DNA binding transcription factors (E2F). Many studies have shown that expression of E2F target genes is essential for cell cycle progression and proliferation.[1] Binding to pocket proteins blocks the transactivation domain (TAD) of E2F and hence E2F-dependent transcription. In addition, pocket proteins can recruit chromatin-remodeling complexes to promoters of E2F target genes through their capacity to simultaneously bind E2F proteins and proteins harboring a leucine-x-cysteine-x-glutamic acid motif (LxCxE, where x is any amino acid).[2] Pocket protein chromatin remodeling complexes have been implicated in processes thought to be critical for tumor suppression such as (irreversible) cell cycle arrest in response to antiproliferative signals and oncogene-induced senescence.[3] Thus, both pRB-mediated inhibition of E2F-dependent transcription and pRB-LxCxE–mediated active silencing of E2F target genes were expected to be essential for tumor suppression. Recent studies question this assumption.

To dissect the contribution of different pRB functions to cell cycle control and tumor suppression, pRB mutants have been created in which a specific function was abrogated. Our laboratory and others generated pRB mutants that are unable to bind LxCxE-containing proteins while retaining the ability to inhibit E2F-mediated transcription. Cells expressing these mutant proteins, collectively designated $pRB^{\Delta LxCxE}$, displayed normal arrest in response to serum deprivation whereas arrest in response to DNA damage or expression of a constitutively active RAS oncogene was impaired.[4–6] Importantly, spontaneous tumor formation was not observed in $Rb^{\Delta LxCxE/\Delta LxCxE}$ mice.[4–6] This sharply contrasts with $Rb^{+/-}$ mice, which develop pituitary tumors at an early age, suggesting that interaction between pocket proteins and LxCxE-containing proteins by itself is not crucial for tumor suppression.

The question then arises of whether inhibition of E2F transactivation by pRB is critical for tumor suppression. A recent report from the Dick laboratory describes a pRB mutant, $pRB^{\Delta G}$, that is impaired in binding the transactivation domain of E2Fs via the pocket region and therefore, by current consensus, is incapable of inhibiting E2F-dependent transcription.[7] As recruitment of $pRB^{\Delta G}$ to E2F binding sites in target promoters is prohibited, active repression of E2F target genes seems also ablated, despite the retained ability of this mutant to interact with LxCxE-containing proteins. Remarkably, $pRB^{\Delta G}$, although largely incapable of regulating E2F activity, still acted as a tumor suppressor in mice.

Taken together, these studies show that the loss of pRB's ability to interact with LxCxE proteins or with the E2F transactivation domain does not promote spontaneous tumor formation in mice. In the first case, pRB-E2F interactions are maintained leaving open the possibility that regulation of E2F-mediated transactivation is sufficient for tumor suppression. In the second case, pRB-LxCxE interactions, independent of E2Fs, are maintained. It is therefore possible that these E2F-independent LxCxE interactions represent a third mechanism by which pRB counteracts proliferation and tumorigenesis. On one extreme, this could imply that E2F regulation is totally dispensable for pRB-mediated tumor suppression. Alternatively, it is possible that each of these mechanisms on its own is capable of suppressing tumorigenesis and hence all 3 mechanisms need to be ablated to reveal a tumor susceptibility phenotype (**Fig. 1**).

Is there evidence that pRB–LxCxE interactions independent of E2Fs could

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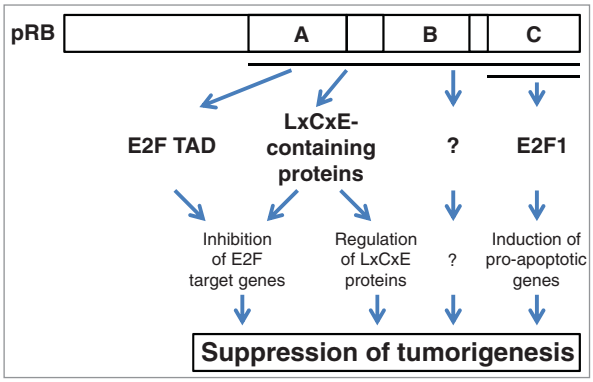

Figure 1. The retinoblastoma protein, pRB, engages in parallel pathways to suppress tumorigenesis. The upper section shows the domain structure of pRB. A, B, and C indicate interacting domains. The middle section indicates interactions between pRB and the E2F family of transcription factors (E2F), LxCxE-containing proteins (harboring a leucine-x-cysteine-x-glutamic acid motif in which x is any amino acid), or unknown proteins. The lower section describes the functional consequences of these interactions.

suppress tumorigenesis? *In vitro* experiments have shown that cell cycle arrest upon γ-irradiation was normal in cells expressing $pRB^{\Delta G}$ but attenuated in those expressing $pRB^{\Delta LxCxE}$, suggesting the involvement of pRB–LxCxE interactions independent of E2F binding.[4,7] Such interactions may also mediate cell cycle arrest in response to activated RAS.[4] LxCxE-dependent interactions between pRB and replication factors (DNA polymerase δ, RFC-p145) have been reported[2] and pRB was found to be recruited to origins of replication in response to DNA damage.[8] Although others have questioned a direct involvement of pRB in DNA replication,[9] these observations may point to a role for pRB in the DNA damage response, possibly providing a tumor suppressive mechanism independent of E2F regulation. The contribution of such a mechanism can be tested by combining the mutations in pRB that abrogate interactions with E2F and with LxCxE-containing proteins, thereby creating $pRB^{\Delta G\text{-}\Delta LxCxE}$, and investigating whether this double mutant protein can suppress development of pituitary tumors in mice.

Should $Rb^{\Delta G\text{-}\Delta LxCxE/+}$ mice remain refractory to spontaneous tumorigenesis, a fourth tumor suppressive activity of pRB must be envisaged that acts parallel to pRB activities relying on binding of E2F and LxCxE. Such a mechanism could involve the interaction of E2F transcription factor 1 (E2F1) and the C-terminus of pRB. This interaction is fundamentally different from the general pRB–E2F interaction that blocks the E2F transactivation domain, is unique for E2F1, and was suggested to specifically function in the induction of proapoptotic genes in response to DNA damage and possibly oncogenic stress.[10] To analyze whether this mechanism can contribute to tumor suppression, it will be interesting to test whether suppression of apoptosis exposes a tumor susceptibility phenotype of the described pRB mutants. One approach is to generate $Rb^{\Delta G\text{-}\Delta LxCxE/+}$ mice overexpressing B-cell lymphoma 2 (BCL2) in the pituitary intermediate lobe. Alternatively, yet unidentified functions of pRB could mediate its tumor suppressor role. Immunoprecipitation experiments using different pRB mutants (defective in E2F- and/or LxCxE-binding) and identification of the interacting proteins by mass spectrometry may help uncover the multitude of tasks that pRB can perform to suppress tumorigenesis.

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